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In-Silico Computational Strategies for Structure-Based Characterization of the Fusion Protein of *Nipah henipavirus* †

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Abstract: Throughout history, viral epidemics of varying frequency and intensity have been responsible for inducing panic and causing widespread damage. The Nipah virus has one of the highest rates of fatalities of any infectious disease in the world. There have been cases when severe respiratory distress has resulted in death, and it is known that these cases can cause encephalitis. The appearance of the virus and its ability to spread is affected by several factors. Several strategies have been created to raise awareness about the need for personal hygiene and enhance surveillance within the contaminated zone. This work aimed to determine the characteristics of a previously unidentified protein linked with the fusion of *Nipah henipavirus* particles. The protein's secondary structure comprises helix, sheet, turn, and secondary coil structures. The protein is a fusion protein. In addition, the estimated Ramachandran plot provided evidence of the accuracy of the modeled protein structure. This accuracy was then verified by the Z-score-based and local model quality evaluation methods. It is possible to think of the protein as a target for developing prospective therapeutic and vaccine candidates directed against the protein to fight viral infections.

Keywords: Nipah virus; protein characterization; Protein stability; GRAVY; viral infection

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1. Introduction

The Nipah virus (NiV), spread by bats and can cause fatal encephalitis in people, has recently been identified in Malaysia, Bangladesh, Singapore, and India [1–3]. It belongs to the order Mononegavirales, which contains other developing lethal zoonotic viruses, including Hendra, Marburg, and Ebola [4]. The virus is thought to be stored naturally in the bodies of Pteropus fruit bats. Humans got NiV from pigs, the intermediate hosts of the virus, in 1998 during the first documented epidemic in the Malaysian town of Sungai [5–7]. Since 2001, the intake of raw date palm sap contaminated with the saliva and excreta of the bats has been reported as the source of yearly NiV outbreaks in various districts of Bangladesh. The first epidemic in India was recorded in Siliguri, West Bengal, in 2001, and it was mainly spread by intimate personal contact or nosocomial transmission. In 2007, a second outbreak was reported in Nadia and West Bengal [7,8]. In a recent NiV epidemic in the Kozhikode region of Kerala, a state in South India, the index patient was said to have been infected by fruit-eating bats [9]. While nosocomial transmission accounted for the vast majority of cases, no clinical or statistical data was provided to confirm the frequency of the illness. The most recent epidemic in Kerala had a death rate of 91%, which is typical of all outbreaks [9,10].

Cell-cell fusion (syncytia) in lung, brain, kidney, and heart tissues is caused by Nipah (NiV) and Hendra (HeV) viruses. This results in encephalitis, pneumonia, and frequent death. Henipavirus infections are characterized by membrane fusion, which is required for viral entry and virus-induced cell-cell fusion [11–14]. Understanding the pathobiology

of henipaviruses relies on elucidating the mechanism(s) of membrane fusion, which may lead to discovering new approaches to creating antiviral therapeutics. Viral attachment (G) and fusion (F) glycoproteins must work together to facilitate membrane fusion in henipaviruses. Current theories of henipavirus fusion propose that F is released from its metastable pre-fusion conformation to promote membrane fusion after NiV or HeV G attachment to its cell surface receptors [11,15–18]. The selected protein for this study is a fusion protein of *Nipah henipavirus* associated with viral infections. The physicochemical characteristics and anticipated protein structures of the selected protein demonstrated structure-function relationships of the proteins associated with viral infections. Therefore, this protein can be targeted for predicting antiviral drugs and vaccines against the selected protein to combat viral infections.

2. Materials and Methods

2.1. Protein Sequence Retrieval

The protein sequence (GenBank: QBQ56722.1, NCBI accession: QBQ56722) was retrieved in FASTA format from the NCBI protein sequence database [19].

2.2. Identification of the Physicochemical Properties

The physicochemical characteristics of the protein were demonstrated by using the ExPASy ProtParam tool [20] and the SMS (v.2.0) program [21].

2.3. Secondary Structure Identification and Assessment of the Selected Protein

The SOPMA program [22] was used following the default parameters (output width = 8; the number of conformational states = 4; helix, sheet, turn, and coil; similarity threshold = 8, and window width = 17) to determine the secondary structural parameters. Moreover, the SPIPRED program (v.4.0) [23] was used the determination of the secondary features and topology of the selected protein.

2.4. Determination and Validation of the Three-Dimensional Protein Structure

The three-dimensional structure of the selected protein was anticipated by using the Modeller [24] with HHpred interface [25,26]. Moreover, the PROCHECK program of the SAVES program (v.6.0) [27] was used for the structural validation of the modeled 3D structure of the protein. Also, the ProSA-web program [28] was used to determine the Z-score of the modeled structure for structural assessment.

3. Results and Discussion

3.1. Sequence Retrieval of the Selected Protein

The protein sequence retrieved from the NCBI database contains 546 amino acid residues (Table 1). The fusion protein (accession no. QBQ56722, version no. QBQ56722.1) is found in the QBQ56722 locus of *Nipah henipavirus*.

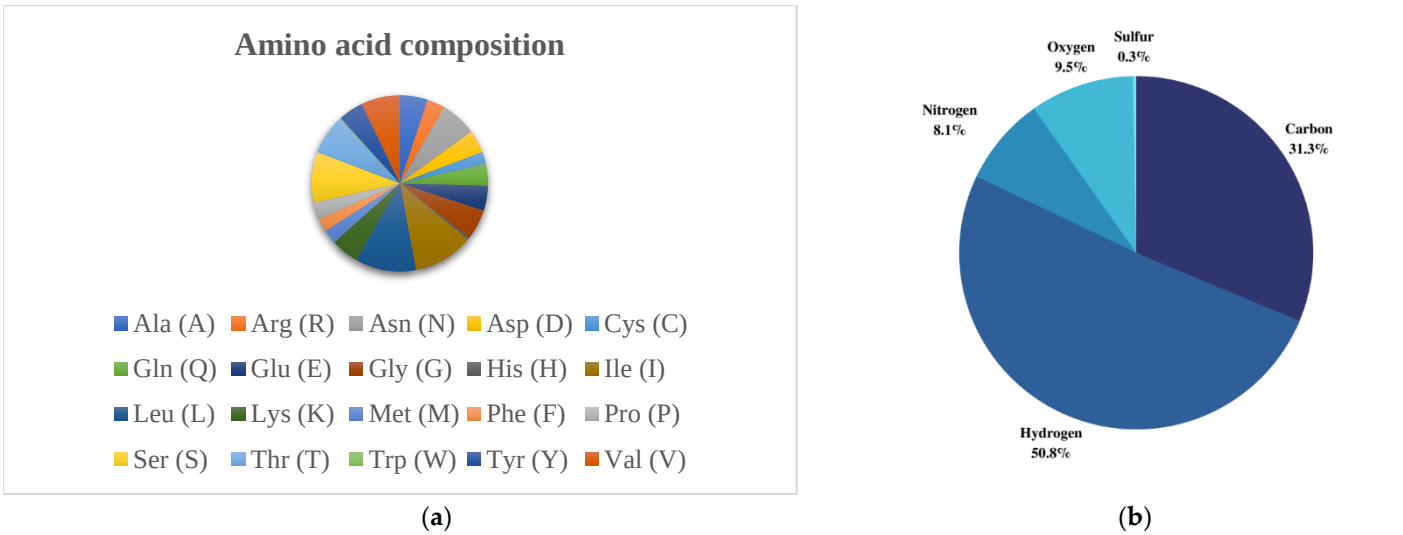
Table 1. Protein retrieval.

Protein Individualities	**Protein Information**
Locus	QBQ56722
Amino acid	546 aa
Accession	QBQ56722
Version	QBQ56722.1
GenBank ID	QBQ56722.1
Source	*Nipah henipavirus*
Organism	*Nipah henipavirus*
FASTA sequence	>QBQ56722.1 fusion protein [*Nipah henipavirus*]

MAVILNKRYYSNLLLLILMISECSVGILHYEKLSKIGLVKGITR
KYKIKSNPLTKDIVIKMIPNVSNMSQCTGSVMENYKTRLNGIL
TPIKGALEIYKNNTHDLVGDVRLAGVIMAGVAIGIATAAQIT
AGVALYEAMKNADNINKLKSSIESTNEAVVKLQETAEKTVY
VLTALQDYINTNLVPTIDKISCKQTELSLDLALSKYLSDLLFVF
GPNLQDPVSNSMTIQAISQAFGGNYETLLRTLGYATEDFDDL
LESDSITGQIIYVDLSGYYIIVRVYFPILTEIQQAYIQELLPVSFN
NDNSEWISIVPNFILVRNTLISNIEIGFCLITKRSVICNQDYATP
MTNNMRECLTGSTEKCPRELVVSSHVPRFALSNGVLFANCIS
VTCQCQTTGRAISQSGEQTLLMIDNTTCPTAVLGNVIISLGKY
LGSVNYNSEGIAIGPPVFTDKVDISSQISSMNQSLQQSKDYIKE
AQRLLDTVNPSLISMLSMIILYVLSIASLCIGLITFISFIIVEKKRN
TYSRLEDRRVRPTSSGDLYYIGT

3.2. Physicochemical Parameters Determination of the Selected Protein

The physicochemical parameters of a protein are defined by the characteristics of its constituent amino acids. The alpha-carbon unit of all amino acids, except for glycine, is asymmetric, indicating that it is connected to four distinct chemical constituents (atoms or atom pairs) [29,30]. Consequently, amino acids, except glycine, can appear in two distinct spatial or geometric configurations (i.e., isomers), which resemble left and right hands [31–33]. ExPASy ProtParam tool identified the physicochemical characteristics of the protein, such as amino acid compositions, atomic composition, and protein half-life calculation (Figure 1). Leucine is the most abundant amino acid (61, 11.2%) compared to others in the amino acid sequence. Moreover, the atomic composition of the protein demonstrated that hydrogen is the most abundant element (4361, 50.8%), following oxygen (817, 9.5%), nitrogen (693, 8.1%), and sulfur (26, 0.3%).



(**a**) (**b**)

Figure 1. Physicochemical parameters of the selected protein. (**a**) The protein contains Ala (28, 5.1%), Arg (18, 3.3%), Asn (36, 6.6%), Asp (23, 4.2%), Cys (12, 2.2%), Gln (22, 4.0%), Glu (25, 4.6%), Gly (30, 5.5%), His (3, 0.5%), Ile (60, 11.0%), Leu (61, 11.2%), Lys (28, 5.1%), Met (14, 2.6%), Phe (13, 2.4%), Pro (17, 3.1%), Ser (51, 9.3%), Thr (40, 7.3%), Trp (1, 0.2%), Tyr (25, 4.6%), and Val (39, 7.1%). (**b**) The atomic composition of the protein as of carbon (2687, 31.3%), hydrogen (4361, 50.8%), nitrogen (693, 8.1%), oxygen (817, 9.5%), and sulfur (26, 0.3%).

The protein has a molecular weight of about 60,280.90 Da (Table 2) with a theoretical pI of 6.08 (6.30*). The protein has the total number of positively charged residues (Arg + Lys), the whole number of atoms, and the absolute number of negatively charged residues

(Asp + Glu) as of 46, 8584, and 48, respectively. As more protein therapies are being developed, many of which have a short plasma half-life, the biotech and pharmaceutical industries are focusing more and more on methods to lengthen that half-life [34,35]. The therapeutic and cost benefits of a longer half-life are apparent. Numerous recognized or in-development biotherapeutics have a short half-life, needing numerous administrations to sustain a therapeutic level over a long period [36–38]. The use of half-life extension techniques permits the production of medicines with enhanced pharmacokinetic and pharmacodynamic characteristics that have a prolonged half-life. Incorporating half-life extension methods into developing numerous biotherapeutics is now standard practice. Various options are available for fine-tuning half-life and adaptation to the desired treatment method and condition [39–42]. The anticipated protein half-life as of 30 h (mammalian reticulocytes, in vitro); >20 h (yeast, in vivo); and >10 h (*Escherichia coli*, in vivo).

Efforts are undertaken to establish a relationship between the metabolic stability of proteins and aspects of their primary sequence and to use weight estimates of instability for a protein of established sequence to determine its resilience properties [43–46]. Proteins may be evaluated for viability in vitro using the 'Instability index.' If the index is under 40, the substance will likely be stable in the test tube. It is presumably not sustainable if it is more significant [47–49]. The instability index of the selected protein is 38.05 (less than 40.00), resulting in a stable nature. The aliphatic index measures how much space is taken up by a protein's aliphatic side chains compared to its total volume [50]. The thermal stability of proteins is related to their aliphatic index. Proteins with a high aliphatic index are less likely to denature when heated. Hydrophobicity is a property shared by aliphatic amino acids [50–52]. The aliphatic index of the selected protein is demonstrated as 112.27. GRAVY is the value employed to demonstrate a protein's hydrophobicity. This value is computed by accepting the absolute hydropathy values of all amino acids (aa) and splitting that whole by the entire sequence length [53–56]. The estimated GRAVY of the protein is 0.177.

Table 2. Physicochemical parameters of the selected protein.

Parameters	Values
Molecular weight	60,280.90 Da
Theoretical pI	6.08 (6.30 *)
Total number of positively charged residues (Arg + Lys)	46
Total number of negatively charged residues (Asp + Glu)	48
Total number of atoms	8584
Estimated half-life	(a) 30 h (mammalian reticulocytes, in vitro) (b) >20 h (yeast, in vivo) (c) >10 h (*Escherichia coli*, in vivo)
Instability index (II)	38.05
Aliphatic index	112.27
Grand average of hydropathicity (GRAVY)	0.177

* pI calculated by the SMS v2.0 tool.

3.3. Identification and Validation of the Predicted Secondary Structure of the Selected Protein

In the context of a polypeptide chain, the term "secondary structure" refers to the standard and recurrent spatial configurations of neighboring amino acid residues. Hydrogen bonds between amide hydrogens as well as carbonyl oxygens of the peptide backbone are responsible for its stability. Alpha-helices (α-helices) and beta-structures (β-structures) are the two most important types of secondary structures [57–59]. The SOPMA program

demonstrated that the protein contains alpha helix (239, 43.77%), extended strand (112, 0.51%), beta turn (23, 4.21%), and random coil (172, 31.50%). No Pi helix, beta bridge, bend region, and ambiguous states were present in the protein (Figure 2). The selected protein contains polar, non-polar, aromatic group-containing, and hydrophobic amino acid residues in its structure (Figure 3). Moreover, the sequence plot demonstrated the protein parameters, including the protein's helical, coil, and extracellular properties (Figure 3). The secondary structure of the selected protein is illustrated in Figure 4.

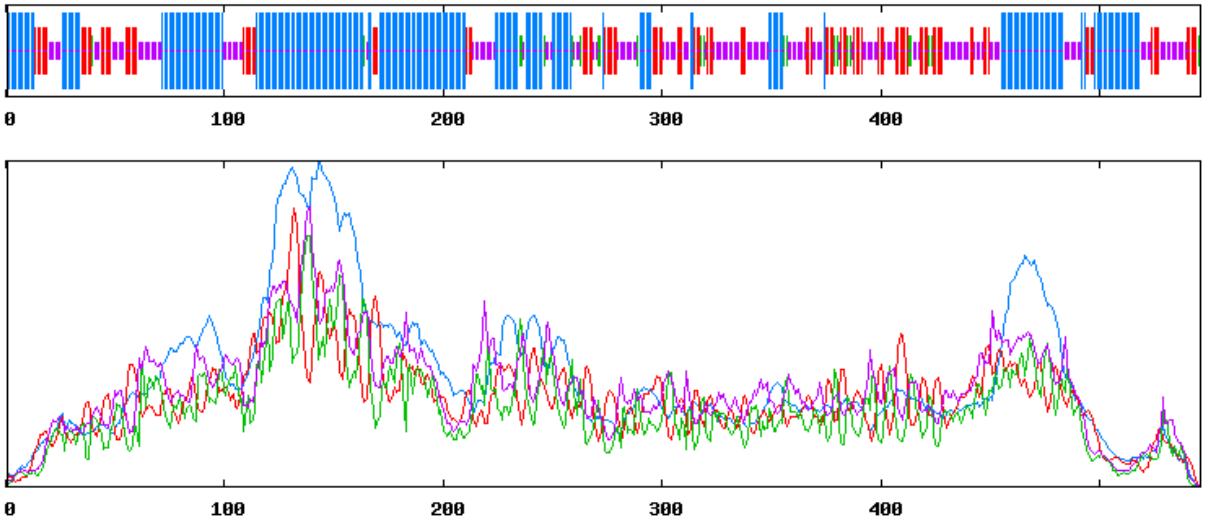
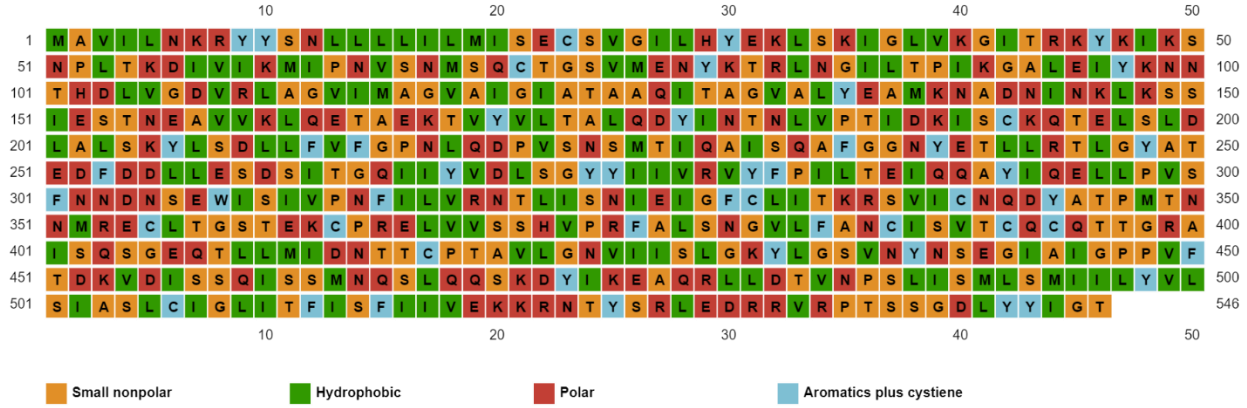
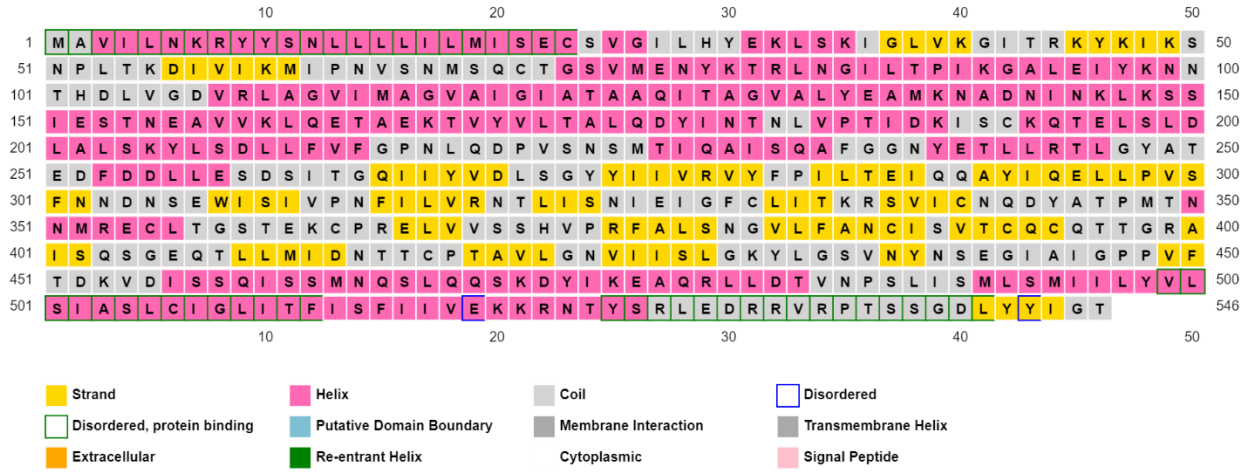


Figure 2. Secondary structural characteristics of the selected protein. The secondary parameters of the selected protein determined the alpha helix (blue color), extended strand (red color), beta-turn (light-green color), and random coil (light-yellow color).



(**a**)



(**b**)

Figure 3. (**a**) Amino acid types of the selected protein, and (**b**) Sequence plot of the selected protein.

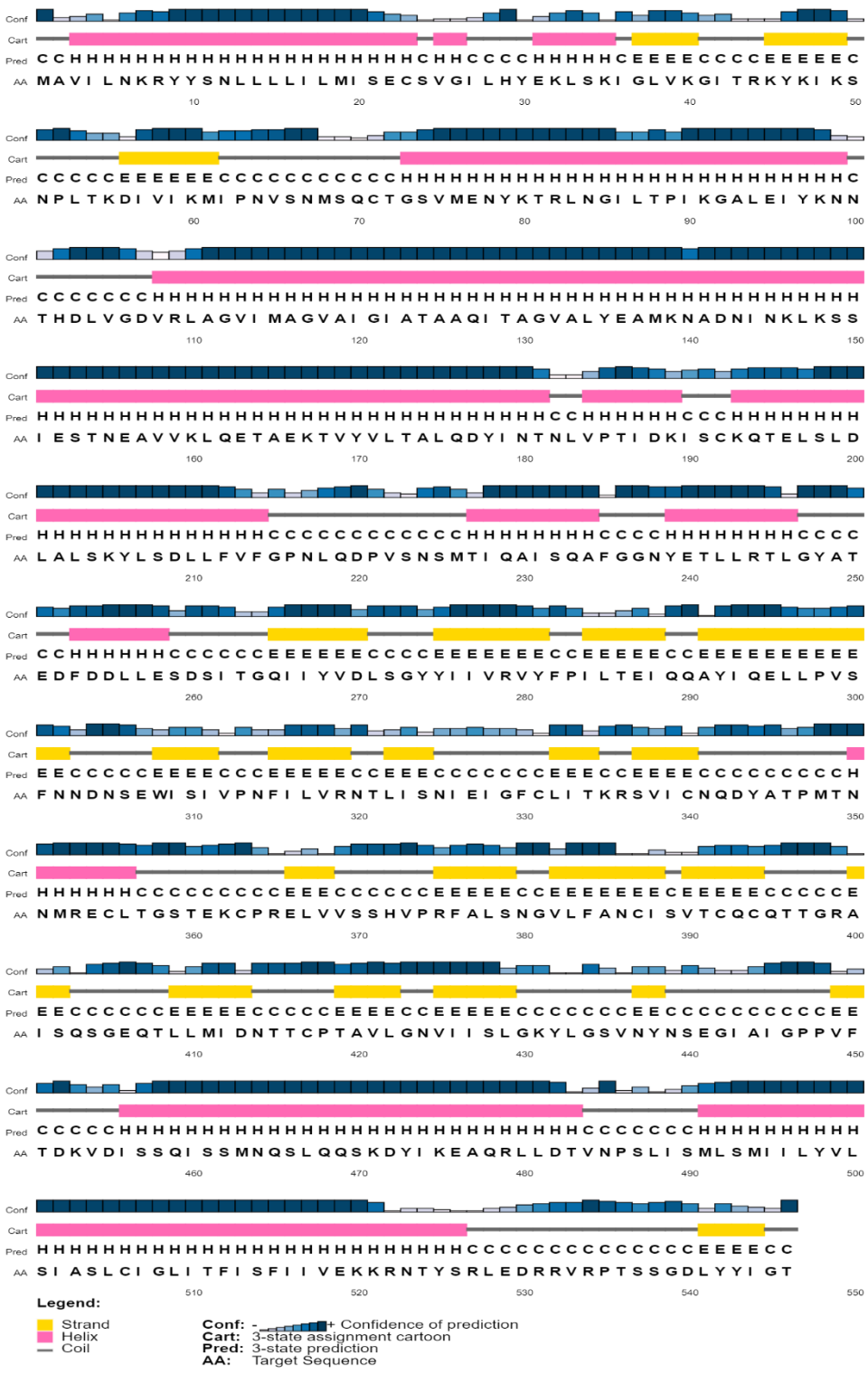


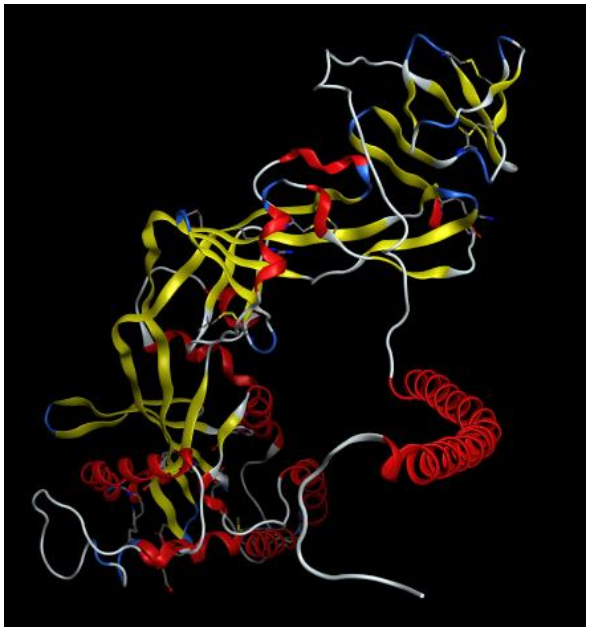
Figure 4. The secondary structure of the selected protein.

3.4. The Three-Dimensional Protein Structure Anticipation and Assessment

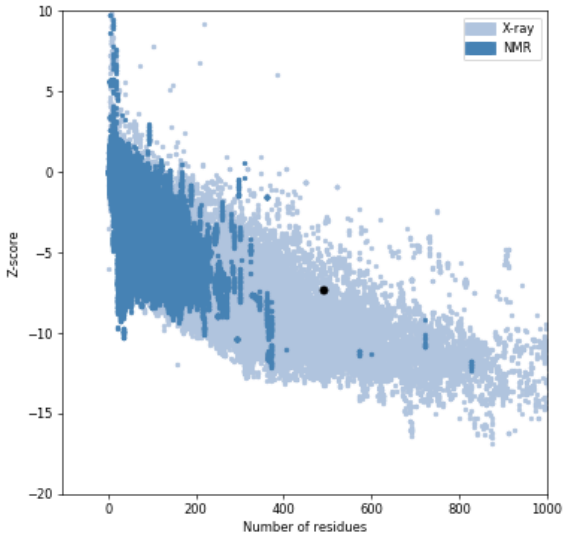
The three-dimensional form of a protein is known as its tertiary structure. One primary 'backbone' polypeptide chain in the tertiary structure comprises one or more protein secondary structures (PSSs) called domains [60–62]. There are a variety of possible interactions and bonds between amino acid side chains. The sequence-structure gap (SSG) is a

significant obstacle in computational biology and chemistry, and protein structure anticipation is one strategy to close this gap. Accurately predicting the structure of a protein is critical since protein structure dictates its function [60,63,64]. The most favored protein templated (HHpred ID: 2B9B_A) was selected for anticipation of the three-dimensional protein structure by the Modeller program with the HHpred interface with the probability of 100%, E-value 2.8×10^{-132}, and target length of 497 (Figure 5).

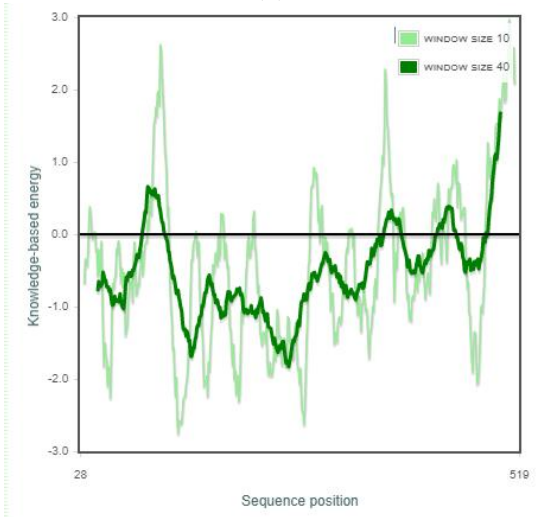
The estimated Ramachandran plot calculations of the selected protein were as residues in most favored regions (411, 91.9%), residues in additional allowed regions (30, 6.7%), residues in generously allowed regions (6, 1.3%), number of non-glycine and non-proline residues (447, 100.0%), and there was no residue in disallowed regions (Figure 5). Moreover, the local model assessment and the overall model quality by Z-score (−7.26) assessed the anticipated protein model quality and validated the structure of the protein.



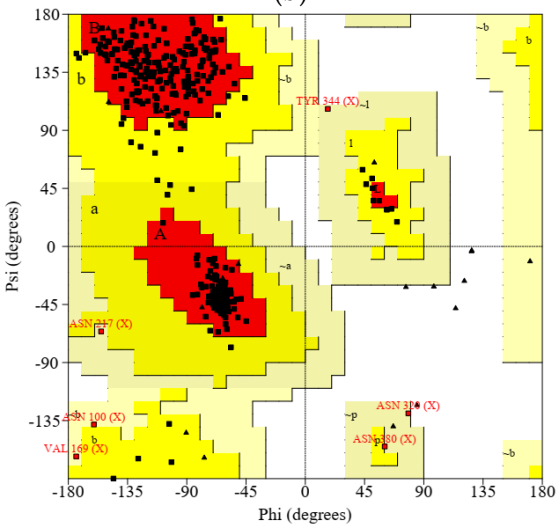
(**a**)



(**b**)



(**c**)



(**d**)

Figure 5. Tertiary structural anticipation and assessment of the selected protein. (**a**) The predicted three-dimensional structure, (**b**) The overall model quality by Z-score (−7.26), (**c**) The local model quality assessment, and (**d**) Ramachandran plot statistics obtained from the SAVES program.

4. Conclusions

NiV has developed as a fatal zoonotic disease. Bats, the natural reservoir of the virus, are adept at viral propagation and human outbreaks continue to be documented routinely. Since bats may be found worldwide, we might expect to see new epidemics in previously unaffected regions. Acute illness progression and a high death rate make a correct diagnosis challenging. The absence of accessible, affordable diagnostic tests and laboratories to process viral samples makes the situation worse. The total caseload is low, and the course of infection is rapid. Thus there is a dearth of investigations into human subjects that might yield effective therapy and prevention. The selected protein's secondary and tertiary characteristics demonstrated the protein structure-based relationships and, therefore, more comprehending properties of the protein. The protein is a fusion protein deeply associated with viral infection. Therefore, the selected protein can be a target for both protein-based drug and vaccine design against the protein to minimize viral infections.

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Conflicts of Interest: The author declares no conflict of interest.

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